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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Group: 1638
Confirmation No.: 3359

Application No.: 10/766,389

Invention: ENHANCED PLANT CELL

TRANSFORMATION BY AD DITION OF HOST GENES INVOLVED IN T-DNA

INTEGRATION

Applicant: Stanton B. Gelvin and Kirankumar S. Mysore

Filed: 01/26/2004

Attorney

Docket: 3220-95461

Examiner: ZHENG, LI

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner of Patents P.O. Box 1450 Alexandria, VA 22313-1450

- 1. I, Stanton B. Gelvin, am a co-inventor of the patent application referenced-above.
- 2. I have read the Office Action mailed March 3, 2007 and reviewed the application as filed and the pending claims.
- 3. The application as filed describes methods by which it was shown that overexpression of H2A leads to increased transformation (see for example, page 9, first and second paragraphs; page 10, third paragraph; and page 11, first paragraph) and describes "histone genes" or "a histone gene" generally, e.g. page 3, fifth paragraph, page 4, first paragraph, and original claims.
- 4. The same methods used to establish the utility of overexpression of *H2A* in increasing cell transformation have now been applied to other histones to demonstrate that

overexpression of histone genes other than H2A in plants, increase transformation efficiency to about two-fold as compared to the wild-type control. For example, histone genes representing core histone groups HTR and HFO, upon overexpression, show increased transformation efficiency as compared to the wild-type control. A summary of the results obtained is provided in attached Exhibit A.

- 4. Using similar vectors, constructs, and methods as taught in the specification, coelectroporation of tobacco BY-2 protoplasts with the *HTA1* gene resulted in a three-fold increase in transformation efficiency as compared to an empty vector. Summary of the results are presented in the attached Exhibit B.
- 5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the Untied States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully Submitted,

Dated: 5/8/07

CHDS01 AOM 396115v1

EXHIBIT A

Primary screening to determine which core histone genes, when over-expressed, can enhance Agrobacterium-mediated transformation of Arabidopsis

Core Histone Gene	Total # of transformed plants screened -	Individual line ID number with increased response to <i>Agrobacterium</i> -mediated transformation	
		Klett 0.1	Klett 0.01
HFO3	20	3; 4; 9; 11; 15; 19	3; 5; 15; 18; 19
HTA1	20	4; 6; 14; 16	6; 9; 14
HTA2	14	5; 11; 13	4; 5; 10; 11; 14
HTA3	18	2; 4; 5; 6; 7; 10;11; 13; 14; 15; 19	1; 4; 5; 7; 9; 10; 11; 13; 14; 15; 17
HTA5	18	1; 5; 6; 11; 15; 16; 18	-
HTA6	5	5	1
HTA6	20	2; 4; 7; 9; 14; 17; 20	6; 9; 17; 20
HTA8	20	-	-
HTA10	-	-	-
HTB1	18	10; 12; 18	5; 8; 10; 15
HTB1	18	5; 8; 10; 15	8; 10; 12; 18
HTB3	6	-	-
HTB5	20	9	-
HTB8	20	6; 18; 20	1; 3; 7; 9
HTB9	9	9	5
HTB10	20	8	8
HTB11	20	13	15
HTR4	20	5; 10; 12	3; 4; 13; 17
HTR11	20	1; 2; 3; 4; 5; 7; 8; 10	7; 8; 16; 19; 20
HTR13	20	6; 11; 13; 16; 17	-

A total of 22 representative core histone genes were tested by expressing cDNAs of these genes under the CaMV 35S promoter in transgenic *Arabidopsis* plants. Infections were performed at a bacterial concentration of 10^5 cells/ml (Klett=0.01) or at 10^6 cells/ml (Klett=0.1).

